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Myosin – a monarch of pigment transport?

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Animals, from insects to mammals, use colouration for a variety of functions including camouflage, photo-protection and seduction of a mate. However, with the exception of well-studied model organisms, such as the mouse, relatively little is known of the molecular basis of colouration and the variation between individuals within a species. Now, an interesting genomic study of the monarch butterfly (*Danaus plexippus*) published in Nature raises the possibility that a common myosin-Va-dependent protein trafficking pathway could regulate colouration by different pigments in different pigmented structures in distantly related species.

The monarch butterfly lays its eggs on the leaves of milkweed plants, from which its caterpillars ingest cardiac glycosides. These accumulate at toxic levels within the caterpillar and adult butterfly. To warn predators of this danger, monarchs display bright orange wing colouration. Interestingly, the monarch population of the Hawaiian Island Oahu is polymorphic for wing colouration and a white morph known as 'nivosus' has been observed there since the 1890s. Classical genetic experiments revealed that the white allele segregates as a single autosomal locus and is recessive to the wild type, meaning that it is maintained at a low frequency (<10%) within the population. In view of the reduced pigmentation, it had been generally considered that the nivosus phenotype most likely results from defects in the biosynthesis of the orange pigment that colours the wild-type monarch wing. However, based on their data, Zhan et al. raise the possibility that pigment transport, rather than production, might be to blame for the white wing colour of nivosus.

Coverage Zhan, S., Zhang, W., Niitepõld, K., Hsu, J., Haeger, J.F., Zalucki, M.P., Altizer, S., de Roode, J.C., Reppert, S.M., and Kronforst, M.R (2014). The genetics of monarch butterfly migration and warning colouration. Nature 514: 317–321.

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To investigate the molecular basis of the nivosus phenotype, Zhan and colleagues sequenced the genomes of 12 Hawaiian monarchs, five white and seven orange wild-type individuals, three of which were first- or second-generation descendants of white monarchs. They then scanned SNP genotypes within the genomic data set (and that of 101 other monarchs from around the world, whose genomes were sequenced as part of a large-scale study aimed at identifying genes linked to the migratory behaviour of North American monarchs published in the same study) for segregation patterns that matched the Mendelian inheritance pattern of the nivosus phenotype. This analysis reports markers in a single gene, DPOGS206617 (sequence available at http://monarchbase.umassmed.edu/tools3/Get_gene.cgi?id=DPOGS206617), that segregate with the white phenotype. In their report, Zhan et al. indicate that this gene encodes a myosin motor protein and suggest that this putative myosin might function in a similar fashion to mammalian myosin-Va in pigment organelle transport in pigment cells (melanosomes in melanocytes).

Myosin-Va is a type V alternative myosin (equivalent to type IX in plants), and this class of highly conserved myosin plays important roles in intracellular transport of organelles (such as melanosomes), mRNA and other cargo (Hammer and Sellers, 2012; Trybus, 2008). In common with other myosins, myosin-V heavy chain contains three hallmark domains (from the N-terminus); a motor/head domain that binds and hydrolyses ATP and allows reversible association with F-actin tracks, a neck domain/lever arm which binds to light chains/calmodulin and amplifies the small ATPase-dependent conformation changes in the motor domain to generate the power-stroke, and a tail domain that allows cargo binding. Typically type V myosins have adaptations to these core domains that facilitate their role as transporters. Firstly, the tail domain contains a series of amphipathic alpha-helices that allow dimerization of the heavy chains. Secondly, the motor domain has a high duty ratio, that is it spends ~80% of the ATPase cycle in high affinity contact with F-actin, meaning that it does not diffuse away from the actin track. Finally, the lever arm is extended and comprises six IQ motifs that can bind three calmodulin light chains and allow the myosin-V dimer to span the 36 nm helical pitch of F-actin. These adaptations allow myosin-V to walk hand-over-hand along actin filaments and thus drag cargo through the cytoplasm. In melanocytes, the tail of myosin-Va also allows its attachment to the melanosome membrane, via interaction with the small GTPase Rab27a and its effector melanophilin, and regulates the transport of melanosomes along F-actin into peripheral cytoplasmic extensions known as dendrites (Evans et al., 2014). Melanosome accumulation in dendrites is essential for their subsequent transfer to keratinocytes and thus skin and hair pigmentation. Consistent with this, mutations in the MYOVA gene (as seen in Griscelli syndrome type I patients and *dilute* mutant mice) cause partial albinism (a.k.a. pigment dilution) due to perinuclear melanosome clustering and defects in the transfer of melanosomes from melanocytes to keratinocytes (Hume and Seabra, 2011).

However, in spite of the suggestion that the DPOGS206617 might encode a myosin motor protein, sequence analysis reveals that the similarity between the predicted DPOGS206617 protein and other myosins is rather limited. Briefly, DPOGS206617 encodes a 360 residue protein that appears to contain three IQ motifs (http://prosite.expasy.org/). While the presence of tandem IQ motifs supports the idea that DPOGS206617 could represent a novel pigment transporting myosin, the finding that it lacks an obvious myosin motor domain argues strongly against this. Although IQ motifs are characteristic of the lever arm of myosins they function in Ca^{2+}/calmodulin signalling and are also found in a number of other types of protein, for

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example neuronal growth proteins, voltage operated channels, phosphatases and Ras GTPase-activating protein (Bahler and Rhoads, 2002). Thus it is far from clear that DPOGS206617 protein regulates pigmentation in the monarch by an analogous mechanism to that of myosin-Va in mouse. Indeed searching the monarch genomic database with mouse myosin-Va protein sequence reveals the existence of another gene DPOGS212512 (http://monarchbase.umassmed.edu/tools3/Get_gene.cgi?id=DPOGS212512) whose predicted product is a 2005 residue protein that shares 47% amino acid identity with murine myosin-Va-related protein, myosin-Vb, and contains all of the type V myosin hallmark domains. It may be of interest in future to investigate the function of this protein in monarch pigmentation even though it is clearly not the gene responsible for albinism in the nivosus mutant.

Nevertheless, this raises interesting questions as to the function of this novel IQ domain containing protein and the mechanism by which it might regulate pigmentation in the monarch butterfly that should be the subject of future research. Furthermore, it will be of interest to know how the mutation(s) underlying the nivosus phenotype affect the function of the DPOGS206617 protein as these were not revealed by Zhan et al. in their report.

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Exploring the molecular basis of monarch butterfly color pattern variation

A response to A. Hume's 'Myosin - a monarch of pigment transport?'

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Dear Editor,

The monarch butterfly, *Danaus plexippus*, sequesters toxic cardiac glycosides from its milkweed host plant as a larva and then uses these compounds to defend itself from bird predators as an adult (Brower and Glazier, 1975; Reichstein et al., 1968). Like other examples of warning coloration, the bold orange wing pattern of *D. plexippus* helps deter predators by enhancing predator learning and distinguishing it from co-occurring palatable species. Wing pattern also serves a central role in mediating mimicry between *D. plexippus* and the viceroy butterfly, *Limenitis archippus* (Ritland and Brower, 1991). Across most of its range, the monarch displays a largely similar orange wing pattern, although there is variation in wing size, shape, and hue (Altizer and Davis, 2010; Davis et al., 2012; Dockx, 2007). However, on the Hawaiian Island of Oahu, there is a rare, 'white' form of *D. plexippus*, the *nivosus* morph, that dates back to the 1890s (Vane-Wright, 1993) and has existed at a frequency ranging between 1% and 8% (Stimson and Kasuya, 2000). Individual white monarchs have been reported from various locations across the broad geographic distribution of *D. plexippus* (Vane-Wright, 1993), but on Oahu, the *nivosus* morph is maintained as a stable polymorphism. Previous work by John Stimson and colleagues has explored factors that may maintain this polymorphism as well as its Mendelian genetics (Stimson and Berman, 1990; Stimson and Kasuya, 2000; Stimson and Meyers, 1984), showing the *nivosus* phenotype segregates as a simple autosomal recessive trait.

Recently, Zhan et al. (2014) performed a comprehensive population genomic analysis of 101 *Danaus* genome sequences to explore the genetic basis of migration and color pattern variation in the monarch butterfly. As part of this analysis, we sequenced a number of Hawaiian monarchs, including white and orange samples and two F1 offspring. By scanning the genomes for allelic patterns consistent with the known Mendelian genetics, and then testing additional white and orange specimens reared or collected between 1984 and 1991, our analysis led us to a region centered on the gene DPOGS206617, which I annotated as a myosin gene. My original annotation was based on (i) the presence of clustered IQ motifs, (ii) myosin annotation of some BLAST hits, and (iii) a predicted function of 'myosin light chain binding' of a putative *Drosophila* ortholog (Franke et al., 2006). While many aspects of this gene made it look like a myosin, Hume (2015) has identified important myosin features that are lacking. So, what is DPOGS206617 and how might it generate color pattern variation in the monarch butterfly?

To better pinpoint the identity of DPOGS206617 in relation to myosin genes, I generated a gene tree among IQ motif-containing proteins. The SMART database (http://smart.embl-heidelberg.de/) contains 17 616 IQ motif-containing proteins, but I limited my analysis to the 1,311 arthropod proteins. I downloaded full protein sequences, aligned them with Clustal Omega (Sievers et al., 2011), and inferred a maximum-likelihood tree using FastTree (Price et al., 2009, 2010). I then annotated gene clusters based on information from *Drosophila*

